

## SHORT COMUNICATION

### The chemical defense of the Texas cave harvestman *Chiniquipellobunus madlae*: first report on the family Stygnopsidae and on a North American troglobiont harvestman (Opiliones: Gonyleptoidea)

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**Abstract.** The stygnopsid harvestman *Chiniquipellobunus madlae* (Goodnight and Goodnight 1967) is known from numerous caves in eleven counties in Texas and is a highly adapted troglobiont (Cokendolpher 2004). Adult and juvenile specimens were extracted in methanol, and the major volatile component of their chemical defense secretion was identified as 2-methyl-5-ethylphenol; a minor component was 2, 5-dimethylphenol. Methylethyl phenols and dimethyl phenols have also been identified in other grassatorid Opiliones, but this is the first report of defensive chemistry from a member of the family Stygnopsidae and from a North American troglobiont harvestman.

**Keywords:** Secretion, 2-methyl-5-ethylphenol, 2, 5-dimethylphenol

Harvestmen are well known for their chemical defenses (Gnaspini & Hara 2007). Despite the great diversity within the order Opiliones, many common and phylogenetically significant families of harvestmen remain unstudied for this ecologically important character. As part of an ongoing survey of the order, we describe below the first observations on the chemistry of the repugnatorial secretion of a stygnopsid harvestman, *Chiniquipellobunus madlae* (Goodnight & Goodnight 1967).

Six living specimens of *Chiniquipellobunus madlae*, two adult females and four immatures, collected from six caves in Bexar Co., Texas, USA (Bunny Hole, B-52 Cave, Dos Viboras Cave, MARS Shaft, Pain in the Glass Cave and Platypus Pit), from 21 to 28 October 2008, were dropped into less than 1 ml USP methanol in glass screw-cap vials with Teflon cap liners for extraction. Each individual extract was analyzed separately. GC/MS analysis of the methanol extract was carried out using a Shimadzu model 2010 GC/MS equipped with an RTX-5, 30 m  $\times$  0.25-mm i.d. GC FTIR spectra were obtained using a Hewlett-Packard model 5965B detector interfaced with a Hewlett-Packard 5890 gas chromatograph fitted with a 30 m  $\times$  0.25 mm ZB-5 30 m  $\times$  32 mm i.d. column. The specimens are now preserved in 70% ethanol and will be deposited as vouchers in the collection of the Virginia Museum of Natural History, Martinsville, Virginia.

Two volatile components were observed in the methanol extract of the specimens, in an average ratio of 17:1. The major component (94.2%) was identified as 2-methyl-5-ethylphenol by direct comparison with an authentic sample (Morgan & Pettit 1934). The mass spectrum and chromatographic retention time of the extract component and the authentic sample were identical, as was the vapor phase infrared spectrum. The minor component (5.8%) had a mass spectrum consistent with a dimethylphenol. Comparison with commercial samples of the possible dimethylphenols narrowed the possibilities to 2,4-dimethylphenol and 2,5-dimethylphenol. Since these isomers proved inseparable by gas chromatography under a variety of conditions, the mixture was acetylated (acetic anhydride/pyridine) and compared to acetates of 2,4-dimethylphenol and 2,5-dimethylphenol, which have different retention times. The gas chromatographic retention time of the natural dimethylphenol acetate

matched that of the 2, 5-dimethylphenol acetate. In addition, in the FTIR spectra the frequency of absorption for the carbonyl groups is 1784  $\text{cm}^{-1}$  for the unknown, 1785  $\text{cm}^{-1}$  for the 2,5-dimethyl isomer and 1781  $\text{cm}^{-1}$  for the 2,4-dimethyl isomer.

2-Methyl-5-ethylphenol has been detected before in seven species of harvestmen, namely *Cynorta astora* Goodnight & Goodnight 1942 from Panama (Eisner et al. 1977); *Eucynortula albipunctata* (Pickard-Cambridge 1904) from Costa Rica (Roach et al. 1980); *Pachylodellus goliath* Acosta 1993 from Argentina (Acosta et al. 1993); *Camarana flavipalpi* B. Soares 1945 from Brazil (Machado & Pomini 2008); *Stygnomma spinifera* (Packard 1888) from Florida, USA (Duffield et al. 1981); *Bishopella laciniosa* (Crosby & Bishop 1924) from North Carolina, USA; and *Texella bifurcata* (Briggs 1968) from Oregon, USA (Shear et al. in press). The first two named are in the family Cosmetidae, *P. goliath* is in the subfamily Pachylinae and *C. flavipalpi* in the subfamily Tricommatinae of the family Gonyleptidae, and *S. spinifera* is a member of the family Stygnommatidae. The last two species named are both members of the family Phalangodidae. Gonyleptidae and Cosmetidae are grouped in the superfamily Gonyleptoidea, Phalangodidae in Phalangodoidea, and Stygnommatidae is a family of Samooidea (Giribet & Kury 2007). The phylogenetic relationships of these families are not well resolved. Stygnopsidae, the family to which *Chiniquipellobunus madlae* belongs, is currently placed in Gonyleptoidea, and so the presence of 2-methyl-5-ethylphenol is not surprising and is consistent with this taxonomic and phylogenetic placement. In the two cosmetids, 2-methyl-5-ethylphenol was accompanied by 2,3-dimethylphenol, and in *P. goliath* by 2,3-dimethylphenol, 2,3-dimethyl-5-ethylphenol and three benzoquinones. In *S. spinifera*, the compounds 2,3-dimethylphenol and 2,3-dimethyl-5-ethylphenol were also detected, while 2-methyl-5-ethylphenol was the sole extractable component in *C. flavipalpi*, *B. laciniosa* and *T. bifurcata* (Gnaspini & Hara 2007; Machado & Pomini 2008; Shear et al. 2009). 2,5-dimethylphenol, the minor component in *C. madlae* secretion, was reported earlier by Hara et al. (2005) from *Daguerria inermis* Soares & Soares 1947, like *P. goliath*, a pachyline gonyleptid. In *D. inermis*, 2,5-dimethylphenol was the major component, making up 61.8% of the secretion; other compounds were not identified (Hara et al. 2005).

Machado and Pomini (2008) suggested that the use of 2-methyl-5-ethylphenol might have evolved in parallel in the two superfamilies Gonyleptoidea and Samooidea. The finding that this compound occurs in at least one species of Stygnopsidae, considered basal in Gonyleptoidea, and also in the superfamily Phalangioidea raises the possibility that it evolved in an ancestor of the two superfamilies. More data on secretions in a wider phylogenetic sampling is obviously needed.

Stygnopsids are endemic to Central America, Mexico and southern Texas, USA (Mendes & Kury 2007). The family consists of 35 species grouped in eight genera. Phylogenetically, stygnopsids may be basal in the Gonyleptoidea, sister to the other families in the superfamily (Kury & Cokendolpher 2000); however, a more complete data set for grassatorean harvestmen is presently being compiled by Adriano B. Kury and will likely result in changes in the tree (A. Kury pers. comm. to WAS 2009). *Chinquepellobunus* was originally described in Phalangodidae (Goodnight & Goodnight 1944), then transferred to Stygnopsidae (Goodnight & Goodnight 1945), synonymized with *Hoplobunus* Banks (Goodnight & Goodnight 1953), and revalidated by Cokendolpher (2004). *Chinquepellobunus madlae* is a troglobiont known from many caves in Bandera, Bexar, Comal, Edwards, Kendall, Kerr, Kinney, Medina, Terrell, Uvalde and Val Verde counties in Texas. Cokendolpher (2004) remarks that this exceptionally broad distribution of a highly troglomorphic harvestman, which could never survive surface conditions long enough to move between caves not connected underground, is difficult to accept. He notes that the same caves occupied by *C. madlae* are also home to a variety of species of other cave-adapted arthropods, none of which is found over such a vast area as *C. madlae*. He speculates that we are actually dealing with a superspecies, made up of a number of reproductively isolated populations convergent on the same troglomorphic habitus and thus inseparable by their anatomy (Cokendolpher 2004). Genetic data could resolve this problem. Since our specimens came from six different caves, we mention this possibility. However, all specimens showed essentially identical chemical profiles.

Twenty-one of the 35 described species in Stygnopsidae have been collected in caves, most of them exclusively, though not all of these are real troglobionts. Stygnopsids are presumably predatory, the cave-dwelling species feeding on other arthropods found in their habitat. The chemical defense of only one other troglobiotic species, *Goniosoma spelaeum* (Mello-Leitao 1932), a gonyleptid from Brazil, has been studied. In that case the secretions consisted of two benzoquinones (Gnaspini & Cavalheiro 1998). The retention of chemical defenses in troglobionts is interesting, since predator pressure on these harvestmen might be expected to be relaxed in a cave ecosystem, where predators aside from the harvestmen themselves may be absent. It may suggest that their presence in that habitat and their physical adaptations to it are relatively recent.

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